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Haemoglobin Levels in Normal Infants Aged 3 to 24 Months, and the Effect of Iron

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Burman, D. (1972). *Archives of Disease in Childhood*, **47**, 261. **Haemoglobin levels in normal infants aged 3 to 24 months, and the effect of iron.** From the age of 3 to 24 months, capillary Hb levels were measured on normal term infants who received no medicinal iron from any source. The mean and standard deviations are recorded at 3-monthly intervals. Females had a higher Hb than males when considered as an overall trend throughout the period. There was no effect of social class or weight gain from birth in either sex. Birthweight was significantly related to Hb at 3 months in males only and at no other age. There was no relation between illness and Hb.

Compared with earlier series in England, the level of Hb in infants is generally higher now than previously, particularly in the second year of life. Infants were given either 10 mg iron in the form of colloidal ferric hydroxide daily, or a placebo. Iron raised the Hb in males of social classes I and II, those with a birthweight below 3·18 kg, and those who gained most weight. Iron made no difference to the incidence of infection. In the absence of a proven relation between a low Hb and morbidity in an affluent community, the routine administration of iron or other haematinics to normal term infants cannot be justified.

I: Normal Infants

Though anaemia of infancy is an extremely common finding among admissions to paediatric wards, there is very little information about its frequency in the general population in England in recent years. There is similar lack of information about the usually found Hb levels in well infants. The only recent study is that of MacWilliam (1968). This paper describes the methods used to study Hb levels by a survey of infants aged 3 to 24 months, and the results in those who received no medicinal iron during this period.

Methods

Hb was estimated by the standard cyanmethaemoglobin method, using an EEL Colorimeter and an Acuglobin standard (Ortho Pharmaceuticals Ltd.). Standardization was carried out daily. During the actual survey, the reliability of duplicate measurements was assessed by the method of Mainland (1954) on 200 patients. The SD was 0·14 g/100 ml so that 95% of duplicates will agree within 0·56 g/100 ml. All results in this paper are the average of duplicate observations, taken from a toe of the infants until they were walking and then from a finger. The reliability of capillary Hbs has previously been discussed (Burman, 1971a).

Parents with home addresses in 8 wards in the North West part of the City of Bristol were invited to participate in the survey, provided that baby was born on certain prescribed dates between 15 June 1965 and 28 February 1966. Illegitimate babies, low birthweight infants (less than 2·5 kg), twins, and babies from known 'problem families' were excluded. 873 babies should have been included but only 450 started at 3 months. The reasons for non-participation are shown in Table I, and of the 423, 383 either could not be traced or the parents refused to co-operate. This survey was started at the same time as fluoridation of the water supply was being considered by the city council. The local press was full of articles and reports of speeches decrying 'mass medication' and I believe that this campaign was partly responsible for the large number of parents who objected. The only information available about those who did not participate is the birthweight and sex, and in these respects they did not differ significantly from those who participated. After starting in the survey a further 190 did not complete the full 2 years for the reasons shown in Table I. It will be noticed that 16 children were seen at an anaemia clinic, as it was decided that it was not justified to continue to observe infants with Hb below 9 g/100 ml. Of the 450 infants who started, only 12 (2·7%) were coloured.

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TABLE I

Reasons for Infants Failing to Enter Survey or Leaving Before the Age of 24 Months

Reason	Failed to Enter Survey		Left Survey After Starting	
	No.	%	No.	%
Moved from Bristol	84	20 } 42	46	24 } 41
Gone abroad	19	4	11	6
Untraced	78	18	21	11
Parent objected	202	48	43	23
General practitioner objected	5	1	3	2
'Problem family'	9	2	1	1
Mother working	2	½	4	2
Illness of child	6	1	13	7
To anaemic clinic	—	—	16	8
Ill effects of medicine	—	—	14	7
Other	18	4	18	9
Total	423	100	190	100

Parents were invited to bring their children to the Infant Welfare Clinics on special afternoons for inclusion in the survey. A proforma was completed and capillary blood samples were collected by a technician. If a patient did not attend a clinic, the technician visited the home. This process was repeated every 3 months but only data collected within 3 weeks either side of the predetermined day were included in the results used for analysis. In the intervening months, a health visitor went to the home to collect data about illness and checked the supply of medicine. As will be seen in Part II, at 3 months one-half of the babies were given iron and the remainder a placebo. This paper confines itself to all infants who entered the survey at 3 months, together with all those who continued but did not receive any iron from the survey team or from any other source (the No-Iron group). 12 of the 16 children found to have a Hb below 9 g/100 ml were from this group.

Results

The mean and SDs of Hb concentrations at each 3 months are shown in Table II. These figures were subjected to an analysis of variance which showed that the Hb changes with age are highly significant ($F = 5 \cdot 6874$; d.f. 7/1113; $P < 0 \cdot 001$). The shape of the curve is seen in Fig. 1. There is a rise in Hb between 3 and 6 months followed by a slight fall at 9 months. This is followed by a steady rise until 18 months after which there is a further fall. If the fall of Hb which occurs during the first 2 months of life

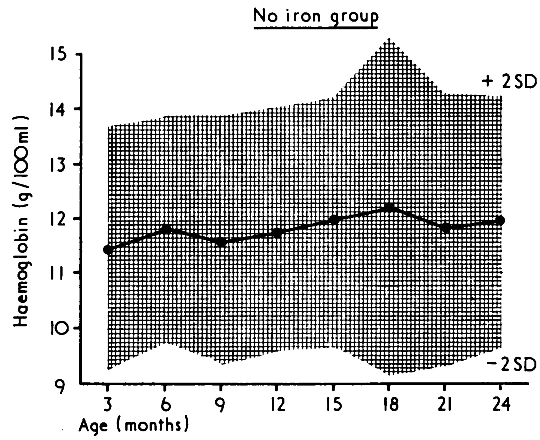

FIG. 1.—*The mean and standardized range of capillary Hb levels in normal infants aged 3 to 24 months.*

TABLE II

Hb Levels in the No-iron Group From 3 to 24 Months

Age (mth)	No. of Observations	Hb (g/100 ml)		
		Mean	SD	Standardized Range (± 2 SD)
3	404	11·54	1·13	13·80—9·28
6	151	11·81	1·00	13·81—9·81
9	113	11·57	1·09	13·75—9·39
12	105	11·75	1·08	13·91—9·60
15	78	11·98	1·15	14·28—9·68
18	82	12·25	1·54	15·33—9·07
21	82	11·85	1·24	14·33—9·37
24	106	11·96	1·16	14·28—9·64

(Gairdner, Marks, and Roscoe, 1952) is called the primary fall, then that which occurs after 6 months could be called the secondary fall, and that which occurs towards the end of the second year of life the tertiary fall. The mechanisms of these changes have previously been discussed (Burman, 1971b).

Since Hb levels vary significantly with age between 3 months and 2 years of age, any study of further factors must take age into account. For example, Table III shows the means and SDs of the Hb levels for males and females at intervals of 3 months. The difference between means for each age group can be compared by a simple 't' test. The mean Hb is higher in females than males in each group except 3 months and 9 months, when they are equal; but the only significant difference occurs at 15 months ($t = 2 \cdot 102$; d.f. $= 76$; $0 \cdot 05 > P > 0 \cdot 01$). This technique, however, generates 8 independent tests which take no account of the overall trend.

A two-factor analysis of variance overcomes this problem. Considering the 'No-iron' group only throughout the age range, subclass numbers are approximately proportional, hence the method described by Snedecor (1962) has been used to study the effect of sex on Hb levels. Table IV shows the detailed results of this analysis. Age *vs.* sex interaction is not significant ($F < 1 \cdot 0$). Therefore, one can assume that the shape of the curve obtained when Hb levels are plotted against age is similar in the two sexes. This variation with age is highly significant ($P < 0 \cdot 001$) and the level of Hb for females is significantly different from that of males ($P < 0 \cdot 01$). From Table III it can be seen that females have higher Hbs than males.

Similar two factor analyses were performed on Hb levels in males and females by age and social class. The social class of the family was determined by the occupation of the father classified according to the General Register Office (1966). Social classes I and II were always considered together and, if social classes were divided into 3 groups, social class III was considered alone and classes IV and V formed a third group. Because the numbers in social classes IV and V were low, they were also combined with social class III to give only two groups. The results of these analyses are shown in Table V. There is no significant effect of social class in either sex and no age–social class interaction. The variation with age is highly significant among the girls, but is not significant for the boys.

Comparable analyses were carried out to see if birthweight (excluding low birthweight infants) made any difference to Hb. The 3 birthweight groupings were less than 3·18 kg, 3·18 to 3·62 kg, and 3·63 kg or more. Birthweight made no significant

TABLE III

Effect of Sex Upon Hb Levels in the No-iron Group

Months	Males Hb g/100 ml			Females Hb g/100 ml			Difference Between Means
	No.	Mean	SD	No.	Mean	SD	
3 (all cases)	206	11·58	1·14	198	11·50	1·12	—0·08
3 (no iron only)	111	11·44	1·15	111	11·55	1·06	0·11
6	76	11·69	1·03	75	11·94	0·96	0·25
9	55	11·58	1·17	58	11·55	1·02	—0·03
12	51	11·67	1·01	54	11·84	1·16	0·17
15	37	11·69	1·16	41	12·23	1·08	0·54
18	43	12·08	1·75	39	12·45	1·28	0·37
21	38	11·71	1·29	44	11·97	1·21	0·26
24	50	11·83	1·19	56	12·08	1·14	0·25

TABLE IV

Two Factor Analysis of Variance to Show Effect of Sex Upon Hb in Infants in the No-iron Group

Variation	Degrees of Freedom	Sum of Squares	Mean Square	F	P
Age	7	49·1406	7·0201	5·2961	<0·001
Sex	1	10·4209	10·4209	7·8617	<0·01
Age × Sex	7	4·9482	0·7069	0·5333	NS
Total between cells	15	64·5098	4·3007		
Subjects within cells	923	1223·4656	1·3255		

TABLE V

Significance of Effect of Social Class on Hb in Infants of No-iron Group

Group	Effect of Age	Effect of Social Class
3 Social Class Groups		
Male	NS	NS
Female	$P < 0{\cdot}001$	NS
Total	$P < 0{\cdot}001$	NS
2 Social Class Groups		
Male	NS	NS
Female	$P < 0{\cdot}001$	NS
Total	$P < 0{\cdot}001$	NS

difference to Hb levels throughout the period of observation in either sex by a two factor analysis of variance; but at 3 months, if all 404 patients examined at 3 months are considered, then there is a significant correlation between birthweight and mean Hb ($F = 3{\cdot}2383$; d.f. 2/401; $0{\cdot}05 > P > 0{\cdot}01$). This significance is retained in the males ($F = 3{\cdot}1279$; d.f. 2/206; $0{\cdot}05 > P > 0{\cdot}01$) but is lost in the females ($F < 1$) though even here the trend is the same (Table VI).

At each age group, the infants were divided into 3 groups according to their weight gain from birth. It was therefore possible to compare those with heavy, medium, or low weight gains, but the division at each age was purely arbitrary, and groupings were selected that made each group approximately equal. A two factor analysis of variance showed no effect of weight gain on Hb in either males or females, or when both sexes were considered together.

Records were kept of both the numbers of illnesses and also the number of days of illness suffered by each child. In order to do this, visits to the family were made by a health visitor every month and particular care was taken to record illnesses occurring at the time of the visit to prevent any illness being included twice. Nisselson and Woolsey (1959) found a recall period for illness of 2 weeks satisfactory, but this was considered to be impractical. Stocks (1949) found that 2 months was too long for patients to remember both major and minor illnesses and thus one month was chosen as a compromise. All the trivial illnesses of childhood were included but serious conditions requiring admission to hospital were not, as by that time the child was withdrawn from the survey. The Hb levels were then related to the numbers of illnesses (none, one, and two or more) and days of illness (none, 1 to 7 days, 8 or more days) by the two factor analysis of variance. There was no significant effect of illness in either sex or in both sexes considered together. Neither did the Hb level make any significant difference to the incidence of infection at any age.

Discussion

The results of any study of Hb levels is dependent upon the method of estimating Hb and this makes comparison of the present study with those previously published difficult. Now, however, the cyanmethaemoglobin method is accepted as an international standard (International Committee for Standardization in Haematology, 1967) and in future, comparisons should be easier. The technique of blood sampling is also important, but the only practical method of studying normal infants is by means of capillary samples. This has been shown to be reproducible in the present work, provided the area to be pricked has been rubbed for 30 seconds beforehand. Capillary samples did not vary significantly from venous samples in the ages under study.

The population sample is equally important and the population considered in Bristol excluded illegitimate babies, low birthweight babies, twins, and babies from known 'problem families'. The Wards of the City that were chosen for the study were known to contain a relatively high proportion of Social Class I and II and were selected to enable a comparison of different socioeconomic groups to be made. Just over a half of the babies who were invited to enter the survey in fact started (Table I), and those who continued until the age of 2 years

TABLE VI

Effect of Birthweight on Hb at 3 Months

Birthweight	All Cases			Males			Females		
	No.	Mean	SD	No.	Mean	SD	No.	Mean	SD
$<3{\cdot}18$ kg	114	11·32	1·29	47	11·22	1·16	37	11·39	1·37
3·18 to 3·62 kg	159	11·59	1·06	79	11·65	1·14	80	11·52	0·98
$>3{\cdot}63$ kg	131	11·67	1·05	80	11·71	1·11	51	11·61	0·95

were only 30% of the total. All infants whose Hb was found to be below 9·0 g/100 ml were excluded. All these factors suggest that the population discussed in this paper was above average in maternal care and social stability.

Despite the fact that this is a selected population, the lower level of the standardized range varies from 9·07 to 9·81 g/100 ml (Table II). MacWilliam (1968), using an identical method of haemoglobinometry, found comparable figures of 10·19 g/100 ml in Buckinghamshire and 9·92 g/100 ml in Newcastle during the second year of life. As a generalization, the lower limit of Hb found in normal infants in this country throughout the ages 3 to 24 months is at its highest 10·0 g/100 ml, and 9·5 g/100 ml would probably be a more realistic figure. Both these estimates are lower than the 11·0 g/100 ml suggested by the World Health Organization (1968) to define anaemia in this age group, but as Murphy and Abbey (1967) have pointed out, the conventional normal range is largely ignored by the experienced clinician.

The other detailed observations made in England on large numbers of infants have been Mackay and Goodfellow (1931) and Horan (1950). Horan used the carboxyhaemoglobin method with hand matching and studied infants born in Birmingham in 1937–9. She eliminated infants who weighed less than 2·72 kg at birth; those who suffered from any neonatal disease or any infection in the first year of life; those whose weight was 10% below the average; those whose mothers were anaemic during pregnancy; and those who were considered to be unhealthy on physical examination. This population is again selected but no information is given about the social background of the infants. Fig. 2 shows that the levels are very similar to the present series but the secondary fall at the end of the first year of life is more profound than that noted 30 years later.

Mackay, too, used the carboxyhaemoglobin method, but the standard used in her original paper (Mackay and Goodfellow, 1931) was later found to be about 7% too low (Mackay, 1933) and so all the figures quoted in this paper will have this 7% added. Her observations were made between 1925 and 1930 in the East End of London. Most were patients attending hospitals for symptoms, but some were attending clinics and some were seen in homes for unmarried mothers. The parents were mostly semi-skilled and unskilled manual workers, frequently with irregular employment. This group is then not comparable with the present series or that of Horan (1950), and it is noticeable that the Hb levels are about 2 g/100 ml

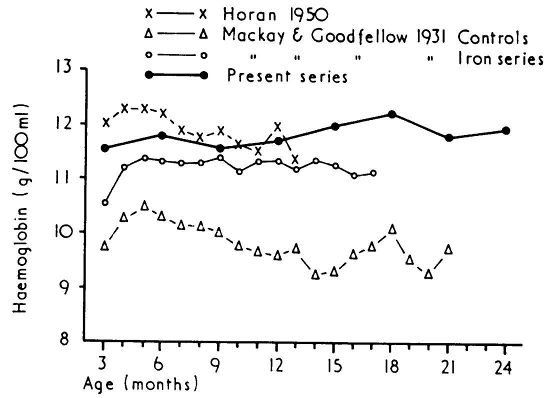

FIG. 2.—*Comparison of mean Hb levels of present series with earlier series in England.*

lower, though the shape of the curve is very similar to that found in Bristol 40 years later (Fig. 2). Mackay and Goodfellow (1931) treated a number of patients with iron for about 5 weeks and then the Hb levels were very similar to the other two series (Fig. 2).

Other less detailed studies in England do not give sufficient detail to allow Hb to be plotted against age, but nevertheless are of value. In 1938, Colver found a mean Hb about 9·9 g/100 ml at both the first and second birthdays in South London. In 1941, a mean of 10·4 g/100 ml was found between 6 and 12 months and 10·05 g/100 ml in the second year of life (Wills *et al.*, 1942). After a further two years of war, the equivalent figures were 10·94 and 10·69 g/100 ml (Medical Research Council, 1945). Davis, Marten, and Sarkany (1960) found a mean of 10·77 g/100 ml in infants attending day nurseries in South London, and Oppé (1964) found means of 11·7 g/100 ml under 5 months, 11·0 g/100 ml for the remainder of the first year of life, and 10·8 g/100 ml in the second year of life in infants attending outpatients in Paddington. Both these authors found lower levels still in West Indian babies. In 1963 in Buckinghamshire, a mean of 12·15 g/100 ml was noted in the second year of life and 12·12 g/100 ml was the equivalent observation in Newcastle (MacWilliam, 1968). The 1963 figures and those in the present investigation are generally higher than most of the earlier observations with the exception of Horan (1950). The higher level is particularly marked in the second year of life.

Comparisons with other comparable countries are even more difficult, but Moe (1965) has reviewed most of the series up to that date. More recent observations from Australia gave a mean figure of

12·2 g/100 ml from 6 to 12 months, and 12·3 g/100 ml in the second year (Lovric, 1970). In Iowa, U.S.A., 11·7 g/100 ml was the mean level from 6 to 18 months, and 12·0 g/100 ml in the last half of the second year (Kripke and Sanders, 1970). The findings of Moe (1965) in Norway showed very similar results to the present series and to the high iron group discussed by Smith and Hunter (1970), but lower figures were found in infants from the lowest socioeconomic group in Chicago by Andelman and Sered (1966), and in South Memphis, Tennessee, U.S.A. (Zee, Walters, and Mitchell, 1970).

The difference between sexes in Bristol is important for it has not been noted before, even when sex has been considered. This difference has been revealed by the statistical methods used in this survey which takes account of the overall trend throughout the period of study. Fry (1961) noted a higher incidence of anaemia in males than females under the age of 10, confirming similar observations in other countries (Woodruff, 1958; Chakravarthy and Vahlquist, 1962; Lovric, 1968). The reason for the higher levels of Hb in females is not known but it may be related to weight gain or birthweight, both of which are usually lower in females. The present study is no exception and the birthweights (in the groupings used in this paper) showed a highly significant difference in the two sexes ($\chi^2 = 15 \cdot 5463$; d.f. 2; P $> 0 \cdot 001$). Even in term infants, birthweight is the most important factor determining iron stores at birth (Burman, 1971b), and Kripke and Sanders (1970) found birthweight to be the only factor, among many they studied, to affect Hb in infancy. A higher birthweight would be expected to produce a higher Hb in males, but in the present investigation, birthweight was only related significantly to Hb at 3 months when the sex difference was negligible (Table III).

As far as weight gain is concerned, this was significantly higher in males at 3, 6, and 9 months. After this age, the trend was the same but the figures did not reach statistical significance. Weight gain did not affect Hb in this series in either sex, confirming an observation of Beal, Meyers, and McCammon (1962). It may still account for the difference between the two sexes, however. The statistical appendices by Bradford Hill to Mackay and Goodfellow (1931) show that up to 6 months birthweight positively influences Hb but later rapid growth is associated with lower Hb. The difference between the sexes in Bristol is most marked in the second year of life (Table III) and this would be compatible with Bradford Hill's conclusions.

The lack of effect of social class in the present work is in contrast to that generally assumed, and that demonstrated by Spence (1960). Lovric (1970) in Australia, Kripke and Sanders (1970) and Owen, Nelson, and Garry (1970) in America confirm the present findings, and deCastro and Miller (1970) found no relation between food cost and anaemia in infants. MacWilliam (1968) suggested an association between social class and Hb but this relation was mainly dependent on 7 girls in social classes I and II from Buckinghamshire with a very high mean Hb. The present series eliminated infants from the lowest socioeconomic group and therefore cannot really be compared with the results of Mackay and Goodfellow (1931) or more recently those of Andelman and Sered (1966).

II: Effect of Iron

At the age of 3 months, alternate infants, registered in birth date order, were given daily either 2 drops of Neoferrum for infants (Crookes Laboratories Ltd.) called mist. nigra, or 2 drops of a placebo called mist. rubra containing sugar, alcohol, sodium citrate, burnt sugar, and permicol red 4880. Two drops of Neoferrum for infants contains 10 mg of elemental iron in the form of colloidal ferric hydroxide. An infant was considered to be having the iron regularly if it was given for two-thirds of the time. This was called the *regular iron* group but if the iron was not given for two-thirds of the time, the child was discarded from the survey. The *no-iron* group previously described was given no medicinal iron from any source but if an infant on the placebo continued in the survey yet did not continue taking the placebo, his observations are included. In fact this was a total of 13 infants and makes no significant difference to any of the results.

Reasons for stopping treatment. 217 infants were given iron only and 218 were given the placebo. 15 infants were given both medicines in error and are excluded from either group. Of a total of 435 patients, 88 stopped the drops before they left the survey because of symptoms attributed to the medicine. 54 of these infants were on Neoferrum, giving an incidence of 25% compared with 16% of those on the placebo. The reasons for stopping are shown in Table VII and are seen to be identical, whether or not iron was present. The frequency of stopping the drops was increased in the iron group ($\chi^2 = 5 \cdot 814$; d.f. $= 1$; $0 \cdot 05 > P > 0 \cdot 01$), but if only definite symptoms are included (the subtotal in Table VII) the difference between the

TABLE VII

Reasons for Stopping Drops in 217 Infants Given Neoferrum and in 217 Infants Given Placebo

Reasons for Stopping Drops	On Neoferrum		On Placebo	
	No.	%	No.	%
Constipated	13	24	8	24
Diarrhoea	2	4	2	6
Vomiting	4	7	1	3
Refusing feeds	2	4	1	3
Screaming	2	4	2	6
Skin rash	3	6	1	3
Subtotal	26	49	15	44
GP's advice	1	2	1	3
Uncooperative parents	11	20	7	21
Ill mother	2	4	1	3
Lack of supplies	7	13	5	15
Other	7	13	5	15
Total	54	100	34	100

placebo and Neoferrum is just not significant ($\chi^2 = 3\cdot314$; d.f. $= 1$; $0\cdot10 > P > 0\cdot05$). These findings are very similar to the incidence of side effects when ferrous salts are used to treat adult blood donors (Sölvell, 1970).

Comparison of regular iron and no-iron groups. The 2 groups were compared by sex, the age of mixed feeding, the use of iron-containing milks, the race of the mother and whether she had had any special training in child care, the occupation of the father, the number of children under 15 in the family, the amount of money spent on food at the beginning of the survey, and the number of children by different social classes. In none of these factors was there a significant difference. Birthweights in the 2 groups are compared in Table VIII and show a significant excess of heavy born infants in the *regular iron* group (the 4·1–4·5 kg groups are added together so that $\chi^2 = 11\cdot846$; d.f. $= 4$; $0.05 > P > 0\cdot01$). This excess occurs in the males only ($\chi^2 = 12\cdot650$; d.f. $= 3$ (under 3·18 kg considered as one group); $0\cdot01 > P > 0\cdot001$). The observed weight at 3 months is heavier in the *regular iron* group ($t = 2\cdot2651$; d.f. $= 375$; $0\cdot05 > P > 0\cdot01$) but observed weight at 3 months is highly significantly related to birthweight ($\chi^2 = 60\cdot063$; d.f. $= 4$; $P < 0\cdot001$). The mean weight gain at 3 months in the *regular iron* group was 2·81 kg and this was not significantly different from the *no-iron* group (2·76 kg). Previously birthweight has been shown to affect the Hb at 3 months only and at no other time in the first 2 years. Birthweight, even excluding low birthweight babies, is the most important factor influencing iron stores at birth (Burman, 1971b), and so heavy born babies would be expected to respond least to iron. In fact, the reverse was the case and so differences in response to iron in the *no-iron* group and *regular iron* group are unlikely to be due to the difference in birthweights.

Results

The comparison between Hb levels in infants receiving no iron and those receiving iron regularly is shown in Table IX and graphically in Burman,

TABLE VIII

Birthweight Distribution in Regular Iron and No-iron Groups

	Under 2·72 kg		2·72 kg—		3·18 kg—		3·63 kg—		4·08 kg—		>4·54 kg		Total
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	
Regular iron													
Males	0	0	14	14	34	34	42	42	8	8	1	1	99
Females	6	7	21	26	32	39	18	22	5	6	0	0	82
Total	6	3	35	19	66	36	60	33	13	7	1	1	181
No-iron													
Males	6	5	29	24	47	39	27	22	10	8	1	1	120
Females	5	4	38	30	54	43	20	16	6	5	1	1	124
Total	11	5	67	27	101	41	47	19	16	7	2	1	244

TABLE IX

Hb Levels in Regular Iron and No-iron Groups Throughout Survey

Age (mth)	Regular Iron			No-iron		
	No. of Observations	g/100 ml		No. of Observations	g/100 ml	
		Mean	SD		Mean	SD
3	160	11·55	1·15	222	11·50	1·10
6	152	11·90	1·02	151	11·81	1·00
9	119	11·60	1·13	113	11·57	1·09
12	107	11·76	1·01	105	11·75	1·08
15	91	12·12	1·10	78	11·98	1·15
18	81	12·38	1·37	82	12·25	1·54
21	82	12·23	1·42	82	11·85	1·24
24	86	12·23	1·33	106	11·96	1·16

1971a. By the two factor analysis of variance used previously, these 2 curves differ at the 5% level and it can be seen that most of the difference occurs during the second year of life, particularly towards its end. This difference is confined to males (Table X). In the *regular iron* group, the sex difference found in the *no-iron* group has disappeared so that iron brings the level of Hb in boys up to that of girls but makes no difference to the Hb of girls.

TABLE X

Effect of Sex and Iron on Hb Levels from 6 to 24 Months

Group	Age	Other Factor
		Sex
No-iron	P < 0·001	P < 0·01 (F>M)
Iron	P < 0·001	NS
		Iron
Males	P < 0·001	P < 0·001
Females	P < 0·001	NS
All cases	P < 0·001	P < 0·05

Table XI summarizes the effect of iron upon Hb in different social classes by the two factor analysis of variance, and shows that a significantly different Hb is produced in social classes I and II only and that this effect is confined to males. Reference to the original data shows that Hb is higher in the *regular iron* group. Though there is no relation between social class and Hb when no iron is given (see Part I), the administration of iron produces a significant relation in males ($P < 0\cdot01$) and in both sexes ($P < 0\cdot001$) but not in females.

By the same methods of analysis, iron was found to produce a higher Hb in infants weighing less than 3·18 kg at birth ($P < 0\cdot05$) and in those with the highest weight gain ($P < 0\cdot05$). Both these significances disappeared if each sex was considered separately. In order to determine if the effect in these 2 groups is due to the same infants that have produced the response to iron in males of social classes I and II, correlations between all these factors were sought. There was no significant relation between social class and birthweight in either sex. Birthweight in the groups used in this study was related to sex ($\chi^2 = 15\cdot546$; d.f. $= 2$; $P < 0\cdot001$) but infants weighing less than 3·18 kg were more likely to be female. Weight gain was found to be significantly higher in males in the *no-iron* group at 3, 6, and 9 months and in the regular iron group at 3, 6, 9, 12, 21, and 24 months. In the remaining age groups the trend was in the same direction. Numbers were too small to allow further analysis by social class. Iron produced no significant difference in weight gain in females at any age but in the males weight gain was significantly higher in the *regular iron* group at 24 months ($\chi^2 = 6\cdot939$; d.f. $= 2$; $0\cdot05 > P > 0\cdot01$) and almost so at 21 months ($\chi^2 = 5\cdot778$; d.f. $= 2$; $P = 0\cdot05$ if $\chi^2 = 5\cdot991$). The infants who gain

TABLE XI

Significance of Effect of Sex, Social Class, and Iron in Hb Levels from 6 to 24 Months

Group	Age	Iron
Both Sexes		
Social Class I and II	P < 0·01	P < 0·001
III	P < 0·001	NS
	NS	NS
III, IV, and V	P < 0·001	NS
Males		
Social Class I and II	P < 0·05	P < 0·001
III, IV, and V	P < 0·01	NS
Females		
Social Class I and II	P < 0·05	NS
III, IV, and V	P < 0·01	NS

most weight are therefore males and probably in social classes I and II.

As in the *no-iron* group, the number of illnesses or days of illness made no significant difference to the Hb in those receiving iron in either sex or when both sexes are considered together. There was no difference in the Hb levels of infants having a different number of illnesses between those receiving iron and the *no-iron* group. Hb level at any age made no difference to the incidence of illness in the *regular iron* group and if the *regular iron* group and the *no-iron* groups were combined there was still no relation between illness and Hb.

Discussion

Table XII summarizes the differences produced by iron in the present series. In every case iron produces a higher Hb than in the control group. The major increase is obviously in males in social classes I and II and evidence has already been produced to show that the infants with a heavy weight gain are the same group. The infants with a birthweight below 3·18 kg are not the same infants.

TABLE XII

Groups of Infants Aged 6 to 24 Months Which Show a Significant Increase in Hb

	Significance
Males	$P < 0.001$
Social Class I and II both sexes	$P < 0.001$
Social Class I and II males only	$P < 0.001$
Birthweight under 3·18 kg	$P < 0.05$
High weight gain	$P < 0.05$
All cases	$P < 0.05$

Iron deficiency is not synonymous with a low Hb, but an Hb level which can be raised by iron indicates that the individual is receiving an intake of iron which is inadequate for maximal Hb formation. The failure to raise Hb by iron in healthy individuals may not only be due to a sufficiency of iron, but also to a deficiency of other essential haematinics, so that iron is not the rate limiting factor.

The response to iron has been used to measure the prevalence of iron deficiency in adult women (Garby, Irnell, and Werner, 1969), in adult men and adolescents of both sexes (Natvig, Bjerkedal, and Jonassen, 1963), and in children (Brigety and Pearson, 1970). In all these groups age has a relatively unimportant influence upon Hb, particularly as the adolescents were only up to 13 years old. In infancy, with its rapid growth rate and continually changing diet, age has a considerable effect upon Hb so that administration of iron to individuals with analysis of the change produced is of very limited value. Longitudinal studies in different children, such as has been carried out here and by Moe (1963), is the only possible method, despite the difficulties of comparison with a control group. The two factor analysis of variance used here permits the statistical significance of changes to be assessed while allowing for the variation with age.

In this series, iron raised the Hb in infants with a birthweight between 2·5 kg and 3·18 kg. In view of the influence of birthweight on iron stores (Burman, 1971b), this result is not surprising and it is an extension of the well-known need to supplement the diets of infants less than 2·5 kg born with iron. The effect of iron is, however, small and it would be necessary to show that this difference was not only statistically significant but also clinically significant (Maxwell, 1969) before advising administration of iron to this group.

The group whose response to iron was greatest was males in social classes I and II. This may be due to the fact that their iron requirements are greater than any other group, possibly due to their greater weight gain. Certainly iron raises the males' Hb to the females' level, suggesting that males are iron depleted. Why is this effect confined to social classes I and II? V. A. Lovric, A. T. Lamni, and J. C. Friend (personal communication, 1971) showed that infants under 1 year of age from poor families in Australia actually have a diet containing more iron than their wealthier counterparts, due to a disproportionate intake of iron-enriched cereals. In the present study, social class was not related to Hb in the *no-iron* group (Table V) but in those receiving iron there was a significant relation between Hb and social class in males ($P < 0.01$) and in both sexes ($P < 0.01$), but this relation was not significant in females ($0.10 > P > 0.05$). Where Hb was related to social class, the higher level was found in social classes I and II. These findings all suggest that, if Hb formation in females is limited at all by nutritional factors, iron is not relevant. The Hb level in males is certainly limited, and part of this limitation is due to iron, but another part is due to a factor which is dependent upon social class. This other factor could be folic acid, and there is evidence to suggest that the folic acid status of normal infants in this country is marginal (Vanier and Tyas, 1966).

The administration of iron made no difference to the incidence of infection in this study and there was no relation between Hb and the incidence of infection in the *no-iron* group, the *regular iron* group, or when both groups were considered together. However, infants with a Hb below 9 g/100 ml were excluded, but that only amounted to 12 infants in the *no-iron* group and 4 in the *regular iron* group. The lowest Hb was 7·8 g/100 ml and this infant died a day later from a respiratory infection. Mackay (1928) and Andelman and Sered (1966) found that the administration of iron reduced the incidence of infection but their infants were drawn from poor socioeconomic groups, and the Hb of their controls was considerably lower than in the series reported here. Lovric (1970) found much lower Hbs in infants admitted to hospital than those attending baby health centres and uses this as presumptive evidence of a relation between morbidity and anaemia. Farquhar (1963) produced a significantly higher Hb in the first year of life by administering iron to infants from an above average socioeconomic group, but concluded that there was no evidence that the difference had any real medical significance. Natvig, Vellar, and Andersen (1967) and Garby (1970) have suggested that the normal range of Hb can only be defined when iron deficiency has been eliminated, but Walker (1969) questions the assumption that raised Hb implies that the previous level was suboptimal and there is now evidence that mild anaemia in adult women is beneficial (Elwood *et al.*, 1970). If iron deficiency must be eliminated to define normality, then other deficiencies, such as the folic acid deficiency postulated above, should also be treated to produce the maximal Hb that is possible. Unless it can be clearly shown that maximal Hb is beneficial to infants, efforts to raise the Hb should not be made, and levels above 9·5 or 10 g/100 ml should be considered nonpathological not only at 6 to 8 weeks (O'Brien and Pearson, 1971) but also for the remainder of the first 2 years.

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